Crystal Structure of RNA 3'-Terminal Phosphate Cyclase, an Ubiquitous Enzyme with Unusual Topology

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Background: RNA cyclases are a family of RNA-modifying enzymes that are conserved in eucarya, bacteria and archaea. They catalyze the ATP-dependent conversion of the 3'-phosphate to the 2',3'-cyclic phosphodiester at the end of RNA, in a reaction involving formation of the covalent AMP-cyclase intermediate. These enzymes might be responsible for production of the cyclic phosphate RNA ends that are known to be required by many RNA ligases in both prokaryotes and eukaryotes.

Results: The high-resolution structure of the Escherichia coli RNA 3'-terminal phosphate cyclase was determined using multiwavelength anomalous diffraction. Two orthorhombic crystal forms of *E. coli* cyclase (space group P2(1)2(1)2(1) and P2(1)2(1)2) were used to solve and refine the structure to 2.1 Å resolution (R factor 20.4%; R(free) 27.6%). Each molecule of RNA cyclase consists of two domains. The larger domain contains three repeats of a folding unit comprising two parallel alpha helices and a four-stranded beta sheet; this fold was previously identified in translation initiation factor 3 (IF3). The large domain is similar to one of the two domains of 5-enolpyruvylshikimate-3-phosphate synthase and UDP-N-acetylglucosamine enolpyruvyl transferase. The smaller domain uses a similar secondary structure element with different topology, observed in many other proteins such as thioredoxin.

Conclusions: The fold of RNA cyclase consists of known elements connected in a new and unique manner. Although the active site of this enzyme could not be unambiguously assigned, it can be mapped to a region surrounding His309, an adenylate acceptor, in which a number of amino acids are highly conserved in the enzyme from different sources. The structure of *E. coli* cyclase will be useful for interpretation of structural and mechanistic features of this and other related enzymes.